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DIATOM DIVERSITY OF SPRINGS AND SPRING-FED STREAMS IN VITOSHA NATURE PARK, BULGARIA

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The paper is dedicated to Prof. D. Temniskova
on the occasion of her 80th jubilee

Abstract: The paper presents the first contemporary study of the diatom diversity of the springs, spring-fed streams, peat bogs and mires forming the headwaters of three rivers (Bistrishka River, Boyanska River and Strouma River) in Vitosha Nature Park. Diatom samples were collected from all natural substrata at each sampling site – epilithon, epiphyton and epipelon. A total of 353 taxa (298 species, 53 varieties and 2 forms) belonging to 70 genera were found. The highest number of species was observed in the genera *Navicula* s.l., *Achnanthes* s.l., *Pinnularia*, *Gomphonema* and *Eunotia.* Fifty-nine taxa are new to the Bulgarian algal flora, and 305 taxa are reported for first time for Vitosha Mountain. The dominant structure of the diatom communities was identified and the number of rare and threatened species was assessed. One hundred and seven taxa (30% of all taxa found in the study) are included in the Red List of limnic diatoms of Central Europe. Notes on some interesting taxa are provided.

Key words: Bacillariophyceae, Bulgaria, diatoms, headwaters, mountains, springs.

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INTRODUCTION

High altitude aquatic habitats are one of the most sensitive ecosystems, sustaining specific and rich biodiversity (Cantonati et al. 2006; Kilroy et al. 2006). These habitats determine the water quality, biodiversity and ecological health of the lower stretches of rivers, which depend on the functions provided by the most upper headwater streams (Lowe & Likens 2005). Moreover, high altitude habitats serve as a 'refugia' or 'habitat islands' for many species (Cantonati et al. 2006; Rott et al. 2006).

In Europe, remote high altitude habitats are one of the very few left, which are not fully affected by humans, however they are highly fragile and sensitive to disturbances (Cantonati et al. 2006). In Bulgaria they are extremely endangered ecosystems, threatened by direct anthropogenic impacts, e.g. water abstraction, destruction and fragmentation of habitats, primarily due to forest management activities, development of winter tourism activities and pollution from wastewater discharges.

In general, diatom communities from oligotrophic habitats in mountain areas are understudied and much less known in comparison to those inhabiting anthropogenically affected waters (Lange-Bertalot & Metzeltin 1996). Habitat destruction and euthrophication threaten many diatom species with extinction, especially those which occur in restricted habitats and are found in low frequencies (Wojtal 2009).

The first studies of diatoms in mountain springs, streams and peat bogs in Bulgaria were made in the beginning of the 20th century by Petkoff (1900, 1904, 1905, 1922, 1925) from Vitosha, Pirin and Rila mountains. Later on Kawecka (1974, 1976, 1980a, 1980b, 1981) published diatoms from Malyovitsa stream in Rila Mt. Diatom communities from mountain springs, spring-fed streams and peat bogs of Ograjden Mt. were studied by Temniskova-Topalova & Misaleva (1982). There are also data on the algal flora of the upper catchment area of the Beli and Cherni Osum rivers in Stara Planina Mt. (Kirjakov & Vodenicharov 1986; Vodenicharov & Kirjakov 1987) and from the upper catchment of Mesta River in Rila Mt. (Passy-Tolar et al. 1999). Recently, diatoms from Bulgarian high altitude lakes have been investigated (Ognjanova-Rumenova et al. 2006, 2009, 2011; Ognjanova-Rumenova 2012). This brief enlistment of studies shows the general lack of contemporary data on diatoms from diverse aquatic mountain habitats of Bulgaria. The most recent data on diatoms in the region of Vitosha Mt. are from Strouma River between the villages Bosnek and Chuipetlovo (Ivanov et al. 2006, 2007).

Due to its proximity to the capital, Vitosha Mountain is the most visited tourist site in Bulgaria, on average by more than 2,5 mln tourists per year (Gussev et al. 2005). This results in a high risk of destruction and fragmentation of habitats, which is one of the main threats for diatom diversity in mountain areas. Investigations of oligotrophic habitats in mountain areas in Europe confirm the presense of a rich diatom diversity (Levkov et al. 2005), as well as a high number of endangered and rare species and the existence of new and potentially new taxa (Lange-Bertalot & Metzeltin 1996). Therefore, the main aim of the study was to investigate the diatom diversity in aquatic habitats located in the protected area Vitosha Nature Park, to assess the taxonomic richness, dominant structure and rare and threatened taxa as well.

STUDIED SITES

Vitosha Mountain (311 km^2 total area, highest peak: Cherni Vrah 2290 m a.s.l.), situated in the middle of Western Bulgaria between Stara Planina Mountain and Rila-Rodopi massif, is the first National Park in Bulgaria and on the Balkan Peninsula, declared in October 1934. At present, since year 2000, 270,1 km^2 of the area of Vitosha Mt. were declared as protected area "Vitosha Nature Park". The mountain is rich in waters – from the high plateaus and peat bogs originate many springs, which form the headwaters of the catchments of several rivers (*e.g.* Palakaria, Zhelezhnishka, Bistrishka, Dragalevska, Vladaiska, Boyanska and Strouma).

The investigated sites (springs, streams, peat bogs, mires) form the headwaters of three main rivers – Strouma, Boyanska and Bistrishka, located in the boundaries of Vitosha Nature Park, between 995 m and 2068 m a.s.l. (Fig. 1).

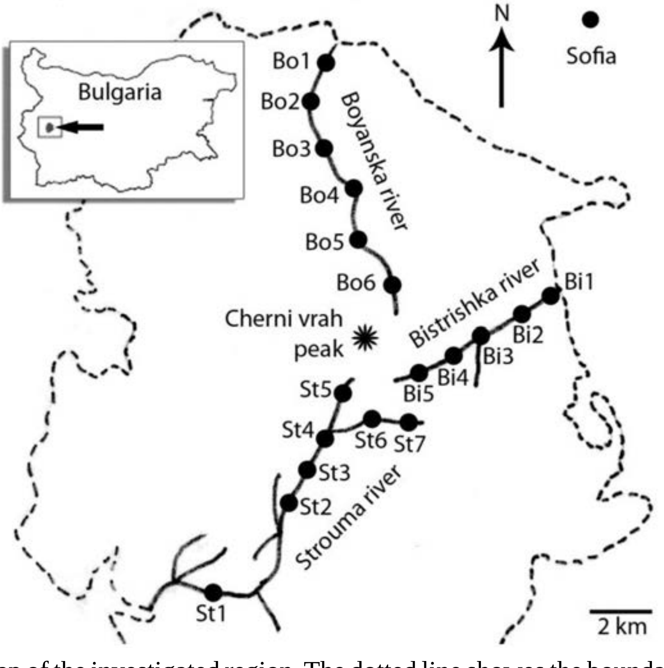

Fig. 1. Map of the investigated region. The dotted line showes the boundaries of Vitosha Nature Park; dots mark the sampling sites (St1-St7: sampling sites from the catchment of Strouma River; Bo1-Bo6: sampling sites from the catchment of Boyanska River catchment; Bi1-Bi5: sampling sites from the catchment of Bistrishka River catchment).

Table 1. List of investigated river catchments, sampling sites, type and number of samples from each site, GPS coordinates, altitude and sampled substrata.

Sampling site	River catchment	Location (gg mm ss.s)	Altitude [m]	Number and type of samples
St1	Strouma	N 42 29 59.5 E 23 12 20,1	995	4 samples (epilithon, epiphython)
St2	Strouma	N 42 31 41,9 E 23 15 05.3	1275	3 samples (epilithon, epiphython)
St3	Strouma	N 42 31 41,9 E 23 15 05,3	1425	3 samples (epilithon, epiphython)
St4	Strouma	N 42 32 17,9 E 23 15 31,1	1485	4 samples (epilithon, epiphython, epipelon)
St5	Strouma	N 42 32 36,7 E 23 15 37,8	1700	4 samples (epilithon, epiphython)
St6	Strouma	N 42 33 04,6 E 23 16 03,9	1988	3 samples (epilithon, epiphython)
St7	Strouma	N 42 30 35,3 E 23 12 52,7	1075	3 samples (epilithon, epiphython)
Bi1	Bistrishka	N 42 34 16,2 E 23 19 47,6	1210	3 samples (epilithon, epiphython)
Bi2	Bistrishka	N 42 34 01,8 E 23 19 06,9	1455	4 samples (epilithon, epiphython)
Bi3	Bistrishka	N 42 33 51,2 E 23 18 52,6	1650	4 samples (epilithon, epiphython)
Bi4	Bistrishka	N 42 33 26,9 E 23 17 49,3	1983	4 samples (epilithon, epiphython, epipelon)
Bi5	Bistrishka	N 42 33 02,5 E 23 18 12,5	2031	4 samples (epilithon, epiphython)
Bo1	Boyanska	N 46 06 40,9 E 26 44 37,6	1060	4 samples (epilithon, epiphython)
Bo2	Boyanska	N 42 37 80,3 E 23 15 30,8	1300	3 samples (epilithon, epiphython)
Bo3	Boyanska	N 42 37 48,2 E 23 15 18,5	1425	4 samples (epilithon, epiphython)
Bo4	Boyanska	N 42 36 51,0 E 23 15 26,6	1650	4 samples (epilithon, epiphython)
Bo5	Boyanska	N 42 33 24,2 E 23 17 52,0	1834	4 samples (epilithon, epiphython)
Bo6	Boyanska	N 42 34 22,6 E 23 16 08,8	2068	4 samples (epilithon, epiphython, epipelon)

MATERIAL AND METHODS

During the period March-November 2008, a total of 66 periphytic diatom samples (54 from lothic habitats – springs, spring-fed streams and 12 from lenthic habitats – mires and peat bogs) were collected from 18 sites (Fig. 1, Table 1):

Boyanska River springs and headwaters – 23 samples from six sampling sites, Bistrishka River springs and headwaters – 19 samples from five sampling sites and Strouma River springs and headwaters – 24 samples from seven sampling sites. The samples were collected from all available natural substrata at each sampling site: stones, cobbles and pebbles (epilithic samples), plants (epiphythic samples: from lothic habitats and from surrounding peat bogs and mires) and epipelithic samples from the sediments' surface. The epiphytic samples were taken from water mosses (*Fontinalis* spp.); peat mosses (*Sphagnum* spp.) and filamentous green algae (*Ulothrix* spp., *Cladophora* spp., *Zygnema* spp.). Sampling was done according to European Standard EN 13946/2003 (*Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*). The samples were fixed *in situ* with 4% formaldehyde. In the laboratory, pretreatment of samples was done according to A.5,2 method of EN 13946/2003 with cold sulfuric acid (H_2S0_4) and potassium permanganate ($KMnO_4$). The cleaned material was mounted on permanent slides with Naphrax©. From each sample two permanent slides were prepared, therefore a total of 132 slides were processed. The materials are stored in the Algal Collection of the Department of Botany, Faculty of Biology, Sofia University "St. Kliment Ohridski".

Light microscopy (LM) was performed on Amplival Carl Zeiss Jena, Nikon Eclipse and Olympus BX51, with 100x oil-immersion objectives, the latter two equipped with digital cameras for light micrographs. Scanning electron microscopy (SEM) was performed with JOEL JSM-5510 operating at 20 kV at the Faculty of Chemistry and Pharmacy (Sofia University "St. Kliment Ohridski").

Diatoms were identified according to Krammer & Lange-Bertalot (1986–1991), Lange-Bertalot & Krammer (1989), Lange-Bertalot (1993, 2001), Lange-Bertalot & Metzeltin (1996), Krammer (1997a, 1997b, 2000, 2002, 2003), Reichardt (1999, 2004), Håkansson (2002), Houk (2003), Nagumo (2003), Werum & Lange-Bertalot (2004) and Bukhtiyarova & Round (1996).

Four hundred valves per slide were counted. The abundance of each taxon in the samples was estimated based on the following categories: rare (one to 5 valves per slide), common (six to 15 valves per slide), subdominant (sixteen to 50 valves per slide) and dominant taxa (above fifty-one valves per slide or the taxon/taxa with the highest relative abundance). The Red List of limnic diatoms (Lange-Bertalot 1996) was used to assess the rare and endangered diatoms.

RESULTS AND DISCUSSION

Taxonomic richness

A total of 353 species, varieties and forms from 70 genera were identified (Table 2). Some of the taxa found are shown on Fig. 2. The diatom flora was predominantly composed of raphid pennate diatoms – 305 taxa (85,3% of all) from 49 genera.

The following genera had the highest number of species: *Navicula* s.l. (including *Naviculadicta* and *Eolimna*) presented with 38 taxa (10,8% of all), *Achnanthes* s.l. (including *Achnanthidium*, *Planothidium*, *Psammothidium* and *Rossithidium*) with 36 taxa (10,2%), *Pinnularia* – 33 taxa (9,4%), *Gomphonema* – 25 taxa (7,1%) and *Eunotia* – 23 taxa (6,5%).

Table 2. List of diatom taxa found on Vitosha Mt. with their distribution (St = Strouma River; Bo = Boyanska River; Bi = Bistrishka River), abundance (1 = rare, 2 = common, 3 = subdominant, 4 = dominant taxa); BG = new taxa to Bulgarian algal flora; RL = Red List (1 = almost extinct, 2 = strongly endangered, 3 = endangered, V = not endangered but in regression, G= presumably endangered, R =extremely rare, D = data insufficient).

№	**Taxa**	**St**	**Bi**	**Bo**	**BG**	**RL**
1	*Achnanthes conspicua* Mayer	2	1	1		
2	*Achnanthes exigua* Grun.	1				
3	*Achnanthes ingratiformis* L-B	1			+	
4	*Achnanthes ricula* Hohn et Hellerman	1				
5	*Achnanthes rupestris* Krasske	1				1
6	*Achnanthes saccula* Carter		1			
7	*Achnanthes silvahercynia* L-B		1	1	+	R
8	*Achnanthes subsalsa* Petersen			1	+	R
9	*Achnanthidium* aff. *atomus* (Hust.) Monnier, L-B et Ector			1		
10	*Achnanthidium kranzii* (L-B) Round et Bukht.	1			+	G
11	*Achnanthidium kryophila* (Petersen) Bukht.		1	1		3
12	*Achnanthidium laevis* var. *austriaca* (Hust.) L-B	1		1	+	
13	*Achnanthidium lineare* Smith	1	1	1		
14	*Achnanthidium minutissimum* (Kütz.) Czarnecki	4	4	4		
15	*Achnanthidium minutissimum* var. *affinis* (Grun.) L-B	1				
16	*Achnanthidium minutissimum* var. *macrocephala* Hust.	2			+	
17	*Achnanthidium pyrenaicum* (Hust.) Kobayasi		1	1		
18	*Achnanthidium subatomus* (Hust.) L-B	4	4	4		
19	*Adlafia bryophila* (Petersen) Moser, L-B et Metzeltin	2	2	1	+	V
20	*Adlafia minuscula* (Grun.) L-B	1	1	1		
21	*Adlafia suchlandtii* (Hust.) L-B			1		
22	*Amphipleura pellucida* (Kütz.) Kütz.	2	1			
23	*Amphora inariensis* Krammer			1		3
24	*Amphora normanii* Rabenhorst	1	1	1		V
25	*Amphora ovalis* (Kütz.) Kütz.		1			
26	*Amphora pediculus* (Kütz.) Van Heurck	1	1	1		
27	*Asterionella formosa* Hassall	1	1	1		
28	*Aulacoseira alpigena* (Grun.) Krammer		4	1		G
29	*Aulacoseira distans* (Ehr.) Simonsen		2	1		G
30	*Aulacoseira distans* var. *nivalis* (Smith) Haworth		1			
31	*Aulacoseira granulata* (Ehr.) Simonsen	1	1	1		

№	Taxa	St	Bi	Bo	BG	RL
32	*Aulacoseira subarctica* (Müller) Haworth	1	1	1		G
33	*Boreozonacola hustedtii* L-B, Kulikovskiy et Witkowski		1			
34	*Brachysira brebissonii* Ross Morph. I	1	1			
35	*Brachysira brebissonii* Ross Morph. II		1	1		
36	*Brachysira intermedia* (Østrup) L-B		1		+	
37	*Brachysira styriaca* (Grun.) Ross		1		+	3
38	*Caloneis* aff. *branderii* (Hust.) Krammer		1			
39	*Caloneis bacillum* (Grun.) Cleve	1	1			
40	*Caloneis branderii* (Hust.) Krammer	1			+	
41	*Caloneis fontinalis* (Grun.) Cleve-Euler	1	1	1	+	
42	*Caloneis pulchra* Messikommer		1	1		
43	*Caloneis sublinearis* (Grun.) Krammer			1	+	D
44	*Caloneis tenuis* (Greg.) Krammer	1	1	1		G
45	*Campylodiscus* sp.1 Ehr.	1				
46	*Cavinula cocconeiformis* (Greg. ex Grev.) Mann et Stickle		1	1		G
47	*Cavinula lapidosa* (Krasske) L-B		1	1		G
48	*Cavinula pseudoscutiformis* (Hust.) Mann et Stickle			1		
49	*Cavinula variostriata* (Krasske) Mann et Stickle	1	1	1		3
50	*Chamaepinnularia schauppiana* L-B et Metzeltin		1		+	
51	*Chamaepinnularia soehrensis* var. *hassica* (Krasske) L-B		1			V
52	*Cocconeis disculus* (Schumann) Cleve		1			R
53	*Cocconeis neodiminuta* Krammer	1	1			R
54	*Cocconeis pediculus* Ehr.	1	1	1		
55	*Cocconeis placentula* var. *euglypta* (Ehr.) Grun.	1	1	2		
56	*Cocconeis placentula* var. *klinoraphis* Geitler	1	2	3		
57	*Cocconeis placentula* var. *lineata* (Ehr.) van Heurck	2	3	2		
58	*Cocconeis placentula* var. *pseudolineata* Geitler	2	2	4		
59	*Cocconeis* sp. 1		1			
60	*Coscinodiscus* sp. 1		1			
61	*Cyclotella meneghiniana* Kütz.	1				
62	*Cyclotella ocellata* Pantocsek	1	1	1		
63	*Cyclotella radiosa* (Grun.) Lemmermann			1		
64	*Cyclotella tripartita* Håkansson		1			
65	*Cymbella affinis* Kütz.		1			
66	*Cymbella aspera* s.l. (Ehr.) Cleve		1	1		V
67	*Cymbella compacta* Østrup	4	1	1		
68	*Cymbella helvetica* Kütz.		1	1		V
69	*Cymbopleura* aff. *subaequalis* (Grun.) Krammer	1		1		G
70	*Cymbopleura* aff. *subcuspidata* Krammer	1		1		
71	*Cymbopleura cuspidata* (Kütz.) Krammer		1			
72	*Cymbopleura naviculiformis* (Auerswald) Krammer	1	1	1		
73	*Cymbopleura* sp.1	1	1	1		

№	Taxa	St	Bi	Bo	BG	RL
74	*Cymbopleura subaequalis* var. *alpestris* Krammer		1		+	
75	*Decussata hexagona* (Torka) L-B	1	1	1		
76	*Denticula tenuis* Kütz.	2	1			
77	*Diadesmis biceps* Arnott		1	1	+	
78	*Diadesmis contenta* (Grun.) Mann	1	1	1		
79	*Diadesmis contenta* var. *parallela* (Petersen) Aboal			1	+	
80	*Diatoma anceps* (Ehr.) Kirchner		1			
81	*Diatoma hyemalis* (Roth) Heiberg	3	4	1		
82	*Diatoma mesodon* (Ehr.) Kütz.	4	4	4		
83	*Diatoma vulgaris* Bory		1			
84	*Diatomella balfouriana* Grev.	1	1	1		
85	*Diploneis* aff. *puella* (Schumann) Cleve		1			V
86	*Diploneis boldtiana* Cleve		1		+	
87	*Diploneis fontanella* L-B		1		+	
88	*Diploneis fontium* Reichardt et L-B	1		1	+	
89	*Diploneis oblongella* (Nägeli ex Kütz.) Cleve-Euler			1		V
90	*Diploneis ovalis* (Hilse) Cleve	1				V
91	*Diploneis peterseni* Hust.		1	1		3
92	*Diploneis pseudoovalis* Hust.	1	1	2		R
93	*Diploneis separanda* L-B			1		
94	*Diploneis subovalis* Cleve		1			
95	*Discotella nana* Hust.	1	1	1		
96	*Encyonema gracile* Rabenhorst	1	4	1		3
97	*Encyonema minutum* (Hilse) Mann	2	1	2		
98	*Encyonema perpusilla* (Cleve) Mann	1	1	1		G
99	*Encyonema silesiacum* (Bleisch) Mann	4	2	2		
100	*Encyonema ventricosum* (Ag.) Grun.	3	1	1		
101	*Encyonopsis cesatii* Krammer		1		+	
102	*Encyonopsis falaisensis* Krammer et L-B		1			G
103	*Encyonopsis* sp. 1		1			
104	*Eolimna minima* (Grun.) L-B et Schiller	2	1	1		
105	*Eolimna tantula* (Hust.) L-B	2	1	1	+	
106	*Epithemia adnata* (Kütz.) Bréb.	2	4			
107	*Epithemia turgida* var. *granulata* (Ehr.) Brun	1				
108	*Eunotia arculus* (Grun.) L-B et Nörpel		1			2
109	*Eunotia bilunaris* (Ehr.) Schaarschmidt	1	1	1		
110	*Eunotia boreoalpina* L-B et Nörpel-Schempp	1	2	4		
111	*Eunotia diodon* Ehr.		1			
112	*Eunotia exigua* var. *exigua* (Bréb. ex Kütz.) Rabenhorst		2	1		
113	*Eunotia flexuosa* Kütz.			1		2
114	*Eunotia glacialis* Meister		1			G
115	*Eunotia groenlandica* (Grun.) Nörpel-Schempp et L-B		2		+	

№	Taxa	St	Bi	Bo	BG	RL
116	*Eunotia implicata* Nörpel, L-B et Alles		1			G
117	*Eunotia incisa* Smith ex Greg.		3			
118	*Eunotia inflata* (Grun.) Norpel-Schempp et L-B		1		+	
119	*Eunotia minor* (Kütz.) Grun.	1	1			
120	*Eunotia monodon* var. *monodon* Ehr.		1	1		2
121	*Eunotia paludosa* Grun.		4	1		V
122	*Eunotia praerupta* var. *praerupta* Ehr.	1	1			3
123	*Eunotia pseudopectinalis* Hust.		1			1
124	*Eunotia rhomboidea* Hust.		1		+	V
125	*Eunotia serra* (s.l.) Ehr.	1	1	1		1
126	*Eunotia soleirolii* (Kütz.) Rabenhorst	2	2	1		G
127	*Eunotia subarcuatoides* Alles, Nörpel et L-B		1	1		
128	*Eunotia tetraodon* Ehr.		1			2
129	*Eunotia triodon* Ehr.		1			1
130	*Eunotia valida* Hust.		2	1		
131	*Fallacia insociabilis* (Krasske) Mann			1		
132	*Fallacia pygmaea* (Kütz.) Stickle et Mann	1				
133	*Fragilaria alpestris* Krasske ex Hust.	2				V
134	*Fragilaria austriaca* (Grun.) L-B	3		1		
135	*Fragilaria bicapitata* Mayer		2	1		
136	*Fragilaria capensis* Grun.	1			+	
137	*Fragilaria capucina* (s.l.) Desmazières		3	2		
138	*Fragilaria capucina* var. *capitellata* (Grun.) L-B	1				
139	*Fragilaria capucina* var. *vaucheriae* (Kütz.) L-B	2	2	1		
140	*Fragilaria construens* f. *binodis* (Ehr.) Hust.	1				
141	*Fragilaria crotonensis* Kitton		1			
142	*Fragilaria fasciculata* (Ag.) L-B		1			
143	*Fragilaria gracilis* Østrup	2	2	1		
144	*Fragilaria leptostauron* (Ehr.) Hust.		1			
145	*Fragilaria perminuta* (Grun.) L-B	1				
146	*Fragilaria polonica* Witak et L-B		2		+	
147	*Fragilaria rumpens* (Kütz.) Carlson	2	2	2		
148	*Fragilariforma virescens* (Ralfs) Williams et Round	1	1	3		V
149	*Frustulia crassinervia* (Bréb.) L-B et Krammer	1	3			V
150	*Frustulia rhomboides* (Ehr.) De Toni		1			
151	*Frustulia saxonica* Rabenhorst		3	1		V
152	*Frustulia vulgaris* (Thwaites) De Toni	1	2	1		
153	*Geissleria decussis* (Østrup) L-B et Metzeltin	1				
154	*Geissleria ignota* var. *palustris* (Hust.) L-B et Metzeltin			1	+	3
155	*Geissleria similis* (Krasske) L-B et Metzeltin	1				V
156	*Gomphonema acidoclinatum* L-B et Reichardt		1	1		
157	*Gomphonema acuminatum* Ehr.	1		1		

№	Taxa	St	Bi	Bo	BG	RL
158	*Gomphonema* aff. *amoenum* L-B	1				
159	*Gomphonema* aff. *anjae* L-B et Reichardt	1		1		
160	*Gomphonema* aff. *lagerheimii* Cleve		1			2
161	*Gomphonema* aff. *variscohercynicum* L-B et Reichardt		1			
162	*Gomphonema amoenum* L-B	1				3
163	*Gomphonema clavatum* Ehr.	2	2	1		
164	*Gomphonema exilissimum* (Grun.) L-B et Reichardt	1	2	1		V
165	*Gomphonema gracile* Ehr.		1	1		
166	*Gomphonema hebridense* Greg.	1	1			V
167	*Gomphonema intricatum* Kütz.	1				
168	*Gomphonema micropus* Kütz.	2	1	3		
169	*Gomphonema minutum* (Ag.) Ag.		1	1		
170	*Gomphonema olivaceoides* Hust.	2	2	2		
171	*Gomphonema parvulum* (Kütz.) Kütz.	1		1		
172	*Gomphonema productum* (Grun.) L-B et Reichardt	1	1	1		
173	*Gomphonema pumilum* var. *elegans* Reichardt et L-B	4	4	4		
174	*Gomphonema rhombicum* Fricke	4	2	4		V
175	*Gomphonema sarcophagus* Greg.		1			V
176	*Gomphonema* sp.	1	2	1	+	
177	*Gomphonema subclavatum* (Grun.) Grun.	1	1	1		
178	*Gomphonema tergestinum* (Grun.) Fricke	3	4	2		G
179	*Gomphonema truncatum* Ehr.	1				
180	*Gomphonema utae* L-B et Reichardt	1	1	1	+	
181	*Hannea arcus* (Ehr.) Patrick	4	3	4		
182	*Hantzschia amphioxys* (Ehr.) Grun.	1				
183	*Hippodonta capitata* (Ehr.) L-B, Metzeltin et Witkowski			1		
184	*Humidophila perpusilla* (Grun.) Lowe et al.	1	2	4		
185	*Hygropetra balfouriana* (Grun.ex Cleve) Krammer et L-B	1	1		+	
186	*Karayevia amoena* (Hust.) Bukht.			1	+	
187	*Karayevia laterostrata* (Hust.) Round et Bukht.	1	1	1		3
188	*Kobayasiella parasubtilissima* (Kobayasi et Nagumo) L-B		3		+	
189	*Kobayasiella subtillissima* (Cleve) L-B		1	1		V
190	*Lemnicola hungarica* (Grun.) Round et Basson	1				
191	*Luticola acidoclinata* L-B	1	1	1		
192	*Luticola* aff. *charlatii* (Peragallo) Metzeltin et L-B	1				R
193	*Luticola goeppertiana* (Bleisch) Mann, Crawford et Mann		1			
194	*Luticola goeppertiana* var. *peguana* (Bleisch) Mann			1	+	
195	*Luticola mutica* (Kütz.) Mann	1	1	1		
196	*Luticola saxophila* (Bock ex Hust.) Crawford et Mann		1		+	R
197	*Mayamaea atomus* var. *permitis* (Hust.) L-B	1	1			
198	*Melosira varians* Ag.	4		1		
199	*Meridion circulare* (Grev.) Ag.	3	3	2		

№	Taxa	St	Bi	Bo	BG	RL
200	*Meridion constrictum* (Ralfs) Van Heurck	1	2	1		
201	*Navicula* aff. *lacunolaciniata* L-B et Bonik	1				
202	*Navicula* aff. *riparia* Hust.		1	1		R
203	*Navicula angusta* Grun.		1			3
204	*Navicula bremensis* Hust.		1			R
205	*Navicula clementis* Grun.	1				
206	*Navicula cryptocephala* Kütz.	1	2	2		
207	*Navicula cryptotenella* L-B	2	1			
208	*Navicula disjuncta* Hust.			1		
209	*Navicula exilis* Kütz.	1	1	1		G
210	*Navicula germainii* Wallace			1		
211	*Navicula gregaria* Donkin	2		1		
212	*Navicula hambergii* Hust.		1			
213	*Navicula harderii* Hust.	2		2		
214	*Navicula jaagii* Meister		1		+	3
215	*Navicula joubaudii* Germain	1	1	1		
216	*Navicula laevissima* var. *perhibita* (Hust.) L-B		1			
217	*Navicula lanceolata* Ehr.	1	2	1		
218	*Navicula lundii* Reichardt			1	+	
219	*Navicula menisculus* Schumann	1				V
220	*Navicula meniscus* Schumann	1				V
221	*Navicula porifera* var. *opportuna* (Hust.) L-B		1			2
222	*Navicula radiosa* Kütz.	2	2			
223	*Navicula recens* (L-B) L-B	1				1
224	*Navicula reichardtiana* L-B			1		
225	*Navicula rhynchocephala* Kütz.	1	1	2		
226	*Navicula* sp.1			1		
227	*Navicula striolata* (Grun.) L-B			1		3
228	*Navicula tripunctata* (Müller) Bory de Saint-Vincent	1	2	1		
229	*Navicula weinzierlii* Schimanski		1	1		R
230	*Naviculadicta difficilima* Hust.		1			G
231	*Naviculadicta digituloides* L-B	1	1	1		3
232	*Naviculadicta multiconfusa* L-B			1	+	R
233	*Naviculadicta seminulum* Grun.	1	1	1		
234	*Naviculadicta suchlandtii* Hust.	1	1		+	V
235	*Naviculadicta tridentula* (Krasske) L-B		1			R
236	*Neidium ampliatum* (Ehr.) Krammer			1		V
237	*Neidium bisulcatum* (Lagerstedt) Cleve		1	1		3
238	*Neidium bisulcatum* var. *subampliatum* Krammer			1		3
239	*Neidium dubium* (Ehenberg) Cleve		1			
240	*Neidium hercynicum* Mayer		1		+	R
241	*Nitzschia acidoclinata* L-B		1	1		

№	Taxa	St	Bi	Bo	BG	RL
242	*Nitzschia agnita* Hust.	1				
243	*Nitzschia amphibia* Grun.	1	1	1		
244	*Nitzschia dissipata* var. *dissipata* (Kütz.) Rabenhorst	1	2	1		
245	*Nitzschia dissipata* var. *media* (Hantzsch) Grun.			1		
246	*Nitzschia fonticola* (Grun.) Grun.	1	1	3		
247	*Nitzschia frustulum* (Kütz.) Grun.					
248	*Nitzschia gracilis* Hantzsch		2	3		
249	*Nitzschia hantzschiana* Rabenhorst	1	2			
250	*Nitzschia inconspicua* Grun.	1				
251	*Nitzschia linearis* var. *linearis* Smith	1	1	1		
252	*Nitzschia linearis* var. *subtilis* (Grun.) Hust.	1				
253	*Nitzschia palea* (Kütz.) Smith	1				
254	*Nitzschia palea* var. *debilis* (Kütz.) Grun.	1				
255	*Nitzschia paleacea* Grun.	1				
256	*Nitzschia perminuta* (Grun.) Peragallo	1	1	1		
257	*Nitzschia pura* Hust.	1		1		
258	*Nitzschia recta* Hantzsch ex Rabenhorst	1	1	1		
259	*Nupela lapidosa* (Krasske) L-B	1	1	3		V
260	*Opephora mutabilis* (Grun.) Sabbe et Wyverman	1	1	1		
261	*Orthoseira roeseana* (Rabenhorst) O'Meara	1	1	1		V
262	*Pinnularia acrosphaeria* Smith			1		
263	*Pinnularia appendiculata* (Ag.) Schaarschmidt		1			
264	*Pinnularia borealis* aff. var. *scalaris* (Ehr.) Rabenhorst		1			
265	*Pinnularia borealis* var. *borealis* Ehr.		1	1		
266	*Pinnularia borealis* var. *scalaris* (Ehr.) Rabenhorst	1	1			R
267	*Pinnularia borealis* var. *sublinearis* Krammer		1	1		
268	*Pinnularia brevicostata* Cleve	1				R
269	*Pinnularia divergentissima* (Grun.) Cleve		1			G
270	*Pinnularia diversa* Østrup			1	+	
271	*Pinnularia eifelana* (Krammer) Krammer		1		+	
272	*Pinnularia esoxiformis* Krammer	1				G
273	*Pinnularia gibba* Ehr.	1	1	1		
274	*Pinnularia intermedia* (Lagerstedt) Cleve			1		V
275	*Pinnularia microstauron* (Ehr.) Cleve		1	1		V
276	*Pinnularia microstauron* var. *nonfasciata* Krammer		1		+	
277	*Pinnularia neomajor* var. *inflata* Krammer		2		+	G
278	*Pinnularia rabenhorstii* (Grun.) Krammer			1	+	
279	*Pinnularia rhombarea* Krammer		1		+	
280	*Pinnularia rupestris* Hantzsch		1			G
281	*Pinnularia schoenfelderi* Krammer			1	+	G
282	*Pinnularia schroederii* (Hust.) Krammer			1		
283	*Pinnularia schwabei* Krasske		1	1	+	

№	Taxa	St	Bi	Bo	BG	RL
284	*Pinnularia scotica* Krammer		2	1	+	
285	*Pinnularia silvatica* Petersen			1	+	
286	*Pinnularia sinistra* Krammer		1			
287	*Pinnularia* sp. 1		1			
288	*Pinnularia* sp. 2			1		
289	*Pinnularia stomatophora* var. *irregularis* Krammer		1		+	
290	*Pinnularia stromatophora* (Grun.) Cleve		1	1		G
291	*Pinnularia subcapitata* Greg.	1	2	2		
292	*Pinnularia subrostrata* (Cleve) Cleve-Euler		1			
293	*Pinnularia viridiformis* morph. II Krammer		1	2		G
294	*Pinnularia viridiformis* var. *viridiformis* Krammer		1			G
295	*Pinnularia viridis* var. *viridis* (Nitzsch) Ehr.	1	2	1		
296	*Placoneis abiskoensis* Hust.		1	1		
297	*Placoneis elginensis* (Greg.) Ralfs	1	1	1		
298	*Placoneis elginensis* var. *cuneata* (Møller ex Foged) L-B	1	1			3
299	*Placoneis ignorata* (Schimanski) L-B		1			
300	*Placoneis paraelginensis* L-B	1	1	1	+	
301	*Planothidium biporomum* (Hohn et Hellerman) L-B	1	1	1	+	
302	*Planothidium ellipticum* (Cleve) Round et Bukht.			1		
303	*Planothidium frequentissimum* (L-B) L-B	2	1	1		
304	*Planothidium lanceolatum* (Bréb.) Round et Bukht.	4	3	2		
305	*Planothidium lanceolatum* aff. var. *boyei* (Østrup) L-B	1				
306	*Planothidium rostratum* (Østrup) L-B	4	2	1		
307	*Psammothidium bioretii* (Germain) Bukht. et Round	1	1	3		V
308	*Psammothidium chlidanos* Hohn et Hellerman	1	1	2		3
309	*Psammothidium curtissimum* (Carter) Aboal		1	2		R
310	*Psammothidium grischunum* (Wuthrich) Bukht. et Round	1	1	1	+	
311	*Psammothidium daonense* (L-B) L-B	1	1	1		G
312	*Psammothidium helvetica* var. *minor* Flower et Jones		1		+	
313	*Psammothidium helveticum* (Hust.) Bukht. et Round	1	1	1		
314	*Psammothidium rechtensis* (Leclercq) L-B			1		
315	*Psammothidium subatomoides* (Hust.) L-B et Archibald	2	1	3		V
316	*Pseudostaurosira* aff. *brevistriata* (Grun.) Williams et Round		1			
317	*Reimeria sinuata* (Greg.) Kociolek et Stoermer	2	1	1		
318	*Rhoicosphenia abbreviata* (Ag.) L-B	1	2	2		
319	*Rhopalodia gibba* var. *gibba* (Ehr.) Müller	3	2			
320	*Rhopalodia gibba* var. *paralella* (Grun.) Holmboe	4				
321	*Rossithidium petersenii* (Hust.) Aboal et al.	1				3
322	*Sellaphora laevissima* (Kütz.) Mann	1		1		
323	*Sellaphora laevissima* var. *perhibita* (Kütz.) Mann			1		R
324	*Sellaphora pupula* var. *pupula* (Kütz.) Mereschkovsky		1	1		

№	Taxa	St	Bi	Bo	BG	RL
325	*Sellaphora rectangularis* (Greg.) L-B et Metzeltin		1			
326	*Stauroneis acidoclinata* L-B et Werum		1	1	+	
327	*Stauroneis* aff. *separanda* L-B et Werum			1		
328	*Stauroneis gracilis* Ehr.			1		V
329	*Stauroneis intricans* van de Vijver et L-B		1		+	
330	*Stauroneis kriegerii* Patrick		1		+	
331	*Stauroneis siberica* (Grun.) L-B et Krammer	1				
332	*Stauroneis smithii* Grun.	1	1			
333	*Stauroneis tackei* (Hust.) Krammer et L-B		1		+	R
334	*Stauroneis thermicola* (Petersen) Lund	1	1			
335	*Stauroneis* sp. 1		1			
336	*Staurosira construens* Ehr.			1		
337	*Staurosira pinnata* var. *intercedens* (Grun.) Hamilton			1		
338	*Staurosira venter* (Ehr.) Hamilton		4	1		
339	*Staurosirella pinnata* (Ehr.) Williams et Round	1	1	2		
340	*Staurosirella pinnata* var. *acuminata* Mayer			1	+	
341	*Stephanodiscus hantzschii* Grun.			1		
342	*Stephanodiscus medius* Håkansson		1			
343	*Stephanodiscus parvus* Stoermer et Håkansson	1		1		
344	*Surirella angusta* Kütz.	1	1	1		
345	*Surirella biseriata* Bréb.		1			
346	*Surirella brebissonii* Krammer et L-B	1				
347	*Surirella brebissonii* var. *kuetzingii* Krammer et L-B	1				
348	*Surirella helvetica* Brun	1				
349	*Surirella linearis* Smith		1			
350	*Surirella spiralis* Kütz.		1			V
351	*Surirella minuta* Bréb.	1	1			
352	*Synedra ulna* (Nitzsch) Ehr.	3	3	4		
353	*Tabellaria flocculosa* (Roth) Kütz.	2	2	1		

Twelve taxa were found in more than 50% of the samples: *Achnanthidium minutissimum* (Kütz.) Czarnecki (in 78,8% of all samples), *Planothidium lanceolatum* (Bréb.) Round et Bukht. (75,8%), *Diatoma mesodon* (Ehr.) Kütz. (69,7%), *Encyonema silesiacum* (Bleisch ex Rabenhorst) Mann (65,2%), *Gomphonema pumilum* var. *elegans* Reichardt et L–B (62,1%), *Humidophila perpusilla* (Grun.) Lowe, Kociolek, Johansen, Van de Vijver, L-B et Kopalová (59,1%), *Gomphonema rhombicum* Fricke (57,6%), *Hannaea arcus* (Ehr.) Kütz. (57,6%), *Achnanthidium subatomus* (Hustedt) L–B (57,6%), *Cocconeis placentula* var. *pseudolineata* Geitler (56,6%), *C. placentula* var. *lineata* (Ehr.) Van Heurck (53%) and *Meridion circulare* (Grev.) Ag. (53%).

The investigated sites forming the headwaters of Bistrishka River had the highest diatom diversity – 244 taxa, followed by the headwaters of Boyanska River with 188 taxa and those of Strouma River with 162 taxa.

Some of the taxa are found in most of the samples, however in low abundance, e.g. *Cavinula lapidosa* (Krasske) L-B, *C. pseudoscutiformis* (Hustedt) Mann et Stickle, *Chamaepinnularia schauppiana* L-B et Metzeltin, *C. soehrensis* var. *hassica* (Krasske) L-B, *Eunotia tetraodon* Ehr., *Gomphonema acidoclinatum* L-B et Reichardt, *G. hebridense* Greg., *Hygropetra balfouriana* (Grunow ex Cleve) Krammer et Lange-Bertalot, *Karayevia laterostrata* (Hust.) Round et Bukht., *Navicula joubaudii* Germain, *Orthoseira roeseana* (Rabenhorst) O'Meara, *Placoneis paraelginensis* L-B and other, which is a typical characteristic of high altitude aquatic environments (Wojtal 2009, Lange-Bertalot & Metzeltin 1996).

Dominant structure

Achnanthidium minutissimum was the most frequent and abundant species in the samples, dominant in 15% of the samples and subdominant also in 15% of the samples. Other abundant (dominant and subdominant) taxa were: *Achnanthidium subatomus, Cocconeis placentula* var. *pseudolineata, Humidophila perpusilla, Diatoma hyemalis* (Roth) Heiberg, *D. mesodon, Encyonema gracile* Rabenhorst, *E. silesiacum* (Bleisch) Mann, *Gomphonema pumilum* var. *elegans, G. rhombicum, G. tergestinum* (Grun.) Fricke, *Hannaea arcus, Melosira varians* Ag., *Meridion circulare, Planothidium lanceolatum* and *Synedra ulna* (Nitzsch) Ehr.

The most frequent and abundant species in the headwaters of Strouma River was *Hannaea arcus;* in the headwaters of Boyanska River such species was *Achnanthidium minutissimum,* whereas in the headwaters of Bistrishka river it was *Gomphonema pumilum* var. *elegans*.

Due to understandable reasons, it is difficult, if possible at all, at present state-of-art to make an objective and detailed comparison between our results and earlier data published on few common diatom taxa from Vitosha Mt. (Petkoff 1922). In general, the taxonomic and dominant structure of the diatom communities of Vitosha Mt. resemble those of Malyovitsa stream in Rila Mt. (Kawecka 1974), where the studied sites were situated at the same altitude range (1000 m – 2000 m a.s.l.). Common taxa for both Rila Mt. and Vitosha Mt. were *Achnanthidium minutissimum, Hannaea arcus, Diatoma mesodon, Meridion circulare* and *Gomphonema pumilum*.

New species to Bulgarian diatom flora and for Vitosha Mt.

Fifty-nine species, varieties and forms from 28 genera were recorded for the first time for the Bulgarian recent diatom flora, which is 17% of all identified taxa (Table 2.). New to Vitosha's algal flora are 305 taxa (Table 2). The high number of new species for Bulgaria and for Vitosha Mt. found in this study, on one hand reflects the lack of sufficient data about recent diatoms in mountain habitats in this region and, on the other hand, results from the high number of new combinations and newly described taxa in the last two decades (*e.g. Gomphonema utae* and *Luticola acidoclinata* L-B).

Red List of limnic diatoms

One hundred and seven taxa (30% of all) found during this study are included in the Red List of limnic diatoms in Germany, respectively Central Europe (Lange-Bertalot 1996). Four of them have a status of 'almost extinct': *Achnanthes rupestris* Krasske, *Eunotia pseudopectinalis* Hust., *E. serra* (s.l.) Ehr., *E. triodon* Ehr. Six taxa are 'strongly endangered': *Eunotia arculus* (Grun.) L-B et Nörpel, *E. flexuosa* Kütz., *E. monodon* Ehr., *E. tetraodon* (Ehr.) Nörpel et L-B, *Gomphonema lagerheimii* (Cleve) Krasske, *Navicula porifera* var. *opportuna* (Hust.) L-B. Nineteen taxa are 'endangered', 25 taxa – 'presumably endangered', 20 taxa – 'extremely rare' and 31 taxa are 'not endangered but in regression' (Table 2).

Some of the rare taxa found were: *Achnanthes silvahercynia* L-B, *Cocconeis disculus* (Schumann) Cleve, *Luticola* aff. *charlatii* (Peragallo) Metzeltin & L-B, *Navicula bremensis* Hust., *Naviculadicta tridentula* (Krasske) L-B and other (Table 2).

Notes on some interesting species

Decussata hexagona (Torka) L-B (Fig. 2: 24) was found in the mires and peat bogs surrounding all of the investigated river catchments, but in low abundance. Until now, the species has been reported for Bulgaria only from Holocene sediments of two mountain peat bogs in the central Sredna Gora Mountains (Stancheva & Temniskova 2006). According to Lange-Bertalot (2001) *D. hexagona* is a rare species, so far known only from Europe, with few localities. The insufficient records of the species in the literature may be due to the limited number of investigations made in mountain habitats. For instance, the species has been found in peat bog and mire samples (Ivanov & Isheva unpubl.) from Osogovo Mountain (Southwestern Bulgaria).

Boreozonacola hustedtii L-B, Kulikovskiy et Witkowski (Fig. 3), previously reported as *Naviculadicta pseudosilicula* (by Lange-Bertalot et Metzeltin in 1996, by Lange-Bertalot et Genkal in 1999 and as *Navicula pseudosilicula* by Hustedt in 1942) has been found in isolated habitats in alpine and boreal regions throughtout the world – Northern Europe, North America, Alaska, Asia (Mongolia). In Central Europe, *B. hustedtii* occurs dispersed in oligotrophic water bodies of the Alps with low electrolyte content **(**Spaulding et al. 2010). So far, for the Balkans, *B. hustedtii* has been reported from Shara Mountain, Macedonia (Levkov et al. 2005) and from few Romanian mountains (Caraus 2012).

Gomphonema aff. *amoenum* L-B (Fig. 4) was recorded only in one site (St4, Strouma River), in an epiphytic sample, as subdominant species, at water temperature of 1,5 C° and altitude of 1485 m a.s.l. The size and shape of the valve in our material differ from the species diagnosis: in *Gomphonema amoenum* valve length is 30–65 μm, valve width is 9–16 μm and striae density is 10–11/10 μm (Krammer & Lange-Bertalot 1991), whereas in *Gomphonema* aff. *amoenum* valve length is 36–66 μm, valve width is 8–12 μm and striae density is 9–12/10 μm.

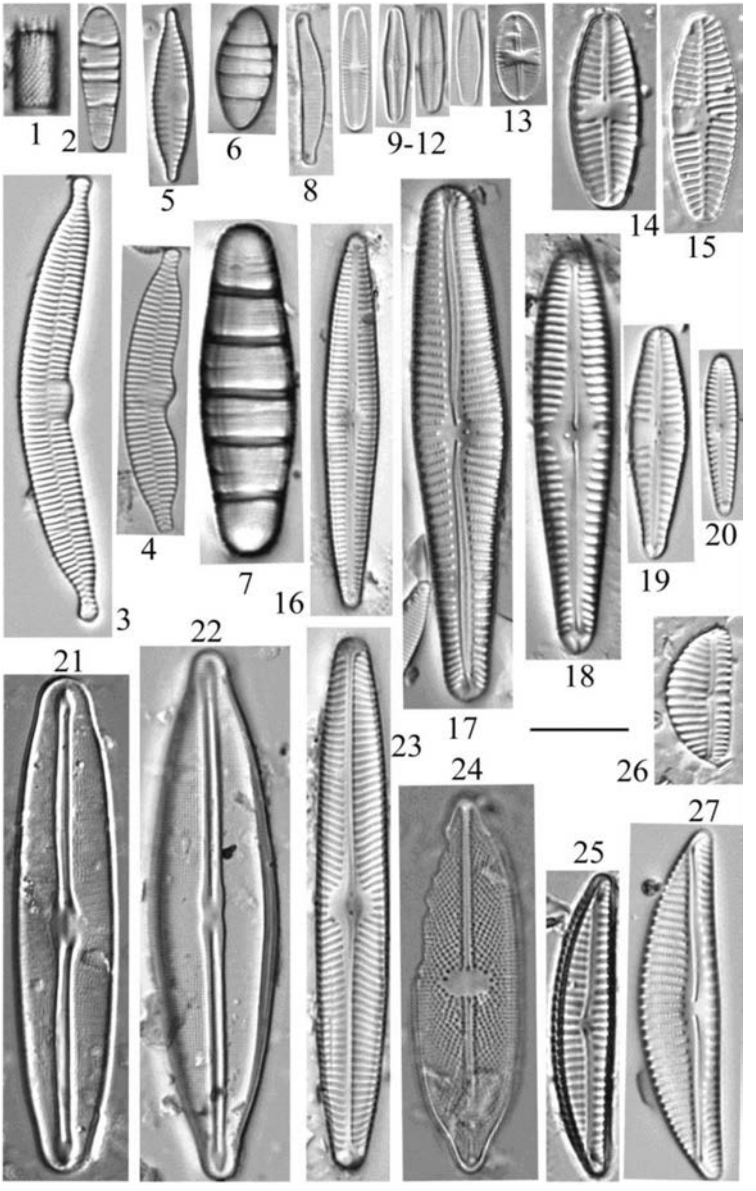

Fig. 2:1–27. Light microscopic micrographs of some diatoms found on Vitosha Mt.: 1 – *Aulacoseira subarctica,* 2 – *Meridion circulare,* 3–4 – *Hannaea arcus,* 5 – *Fragilaria capucina, 6 – Diatoma mesodon,* 7 – *D. hyemalis,* 8 – *Eunotia paludosa,* 9–12 – *Achnanthidium minutissimum*, 13 – *Psammothidium subatomoides*, 14, 15 – *Planothidium lanceolatum*, 16 – *Gomphonema acidoclinatum* 17 – *G. clavatum*, 18 – *G. rhombicum*, 19 – *G. tergestinum*, 20 – *G. pumilum* var. *elegans*, 21 – *Frustulia vulgaris*, 22 – *F. rhomboides*, 30 – *Navicula angusta*, 24 – *Decussata hexagona*, 25 – *Encyonema gracile,* 26 – *E. minutum,* 27 – *E. silesiacum.* Scale bar = 10µm.

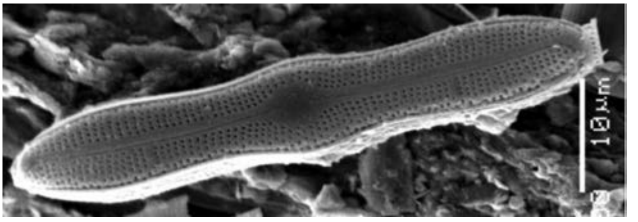

Fig. 3. SEM micrograph of *Boreozonacola hustedtii* L-B, Kulikovskiy et Witkowski.

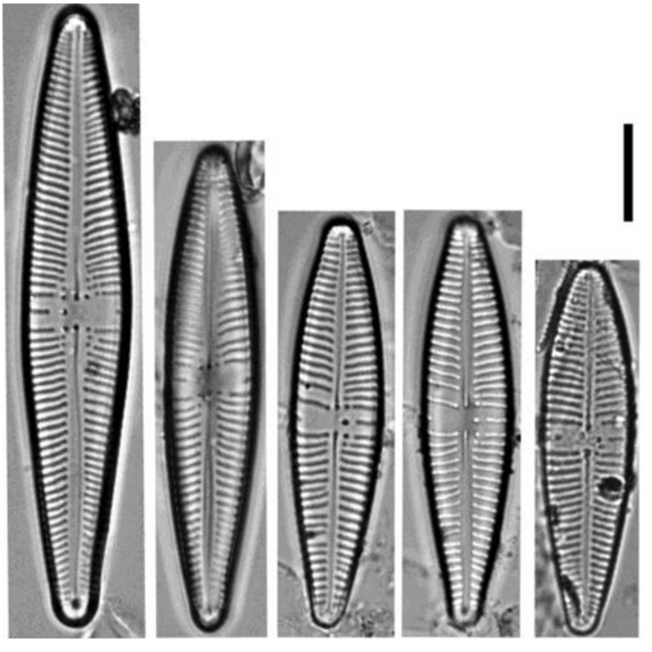

Fig. 4. LM micrographs of *Gomphonema* aff. *amoenum* L-B. Scale = 10 µm.

One species from genus *Gomphonema* (*Gomphonema* sp., Fig. 5) was found in all investigated rivers at 5 sampling sites (epilithic samples, rare and common). It was not possible to identify the species, according to the currently available literature. It was characterized by valve length of 15–35/10 µm, width of 5,0–7,0 µm, striae density of 9–13/10 µm and valves in girdle view 2,0–3,0 µm wide. Observation in SEM showed that the striae are composed of double rows of areolae, with density of 50/10 µm. Only two other species with biseriate areolae are known in the Bulgarian diatom flora: *Gomphonema minutum* (Ag.) Ag. and *G. rhombicum* Fricke. With high probability, *Gomphonema* sp. is a new species to science, which will be further investigated and described elsewhere.

In conclusion, it is possible to state that the diatom flora found in Vitosha Nature Park is characteized by high biodiversity and includes species of high conservation importance. The results obtained during this study could serve as comparative basis for future studies and doubtless show that future more detailed investigations in different regions of Bulgaria will contribute to our knowledge on recent Bulgarian diatom flora.

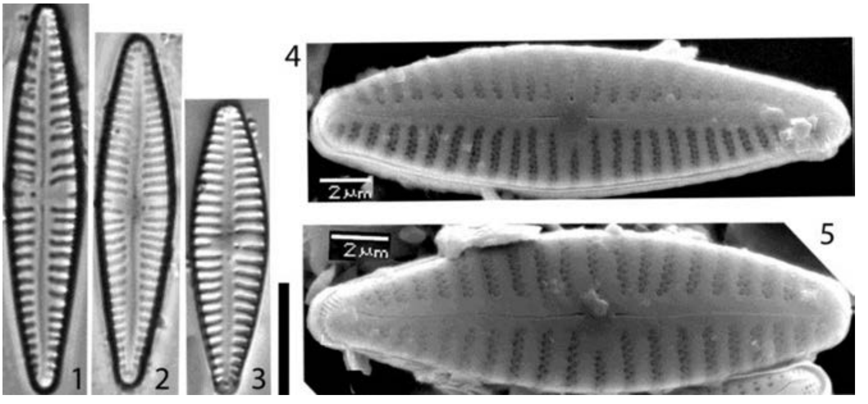

Fig. 5. LM (1–3) and SEM micrographs (4–5) of *Gomphonema* sp. Scale = 10 μm.

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